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Preterm birth after loop electrosurgical excision procedure (LEEP): how cone features and microbiota could influence the pregnancy outcome

A. FREGA[1], M. SANTOMAURO[1], F. SESTI[2], J. DI GIUSEPPE[3], C. COLOMBRINO[1], R. MARZIANI[1], A. CATALANO[1], M. PAVONE[1], C. LEONE[1], M. MALLOZZI[1], E. D'ADAMO[4], A. CIAVATTINI[3], D. CASERTA[1]

[1]Department of Surgical and Medical Science and Translational Medicine, Sant'Andrea Hospital, Faculty of Medicine and Psychology, Sapienza University of Rome, Rome, Italy
[2]Department of Surgical Sciences, School of Medicine, Section of Gynecology and Obstetrics, Tor Vergata University Hospital, Rome, Italy
[3]Woman's Health Sciences Department, Gynecologic Section, Polytechnic University of Marche, Ancona, Italy
[4]Department of Pediatrics, Santissima Annunziata Hospital – Gabriele D'Annunzio University of Chieti, Chieti, Italy

Abstract. – OBJECTIVE: In the last years, the mean age of women who underwent cervical treatment for high-grade cervical intraepithelial neoplasia (CIN 2-3) is similar to the age of women having their first pregnancy. The aim of this study was to evaluate the risk of preterm birth in subsequent pregnancies after loop electrosurgical excision procedure (LEEP).

PATIENTS AND METHODS: From January 2013 to January 2016 the study identified a total of 1435 women, nulliparous, who underwent LEEP for CIN 2-3, and who wished to have their first pregnancy. Before surgery, the lengths of the cervix were calculated by transvaginal sonography. After the treatment, the dimension of the removed tissue was evaluated. During the pregnancy, all women carried out periodic transvaginal sonography and vaginal-cervical swabs.

RESULTS: The average age of patients was 31.96±5.24 years; the interval between the surgical procedure and pregnancy was 12.04±4.67 months; the gestational age at births was 37.53±2.91 weeks. The first vaginal and cervical swab performed during pregnancy was negative in 81.8% of patients. The most prevalent infections were related to *C. Albicans*, *G. Vaginalis*, and *Group B Streptococcus* (GBS). The rate of preterm delivery was significantly higher in women with a minor cervical length.

CONCLUSIONS: The length and the volume of cervical tissue excised have been shown to be directly related to the risk for preterm birth. Furthermore, vaginal infections and their persistence during pregnancy in women with a history of LEEP may be associated with an increased risk for preterm birth, compared with women with no history of LEEP.

Key Words:
CIN, Pregnancy, LEEP, Microbiota.

Introduction

High-grade cervical intraepithelial neoplasia (CIN 2-3) is defined as a precancerous lesion, induced by high-risk human papilloma virus, which could potentially show progression to invasive carcinoma if not removed. The mean age of woman who underwent cervical treatment for CIN 2-3 is similar to the age of women having their first pregnancy. The loop electrosurgical excision procedure (LEEP) is the most common cervical excision procedure currently used. Several meta-analyses of cohorts and large linkage studies[1-7] have analyzed the risk for obstetric complications related to the loss of cervical integrity in subsequent pregnancies. Data from the literature are conflicting[8-11]: several investigations have reported a significantly increased risk for preterm birth after LEEP; in contrast, other researches have shown that, after checking for socio-epidemiological factors associated with the development of

Corresponding Author: Angelica Catalano, MD; e-mail: angelicacatalano87@hotmail.it

CIN, LEEP was not an independent risk factor for preterm delivery. Moreover, it has been recently proposed that local microbial communities may affect the acquisition and the persistence of HPV infection and can induce a local inflammatory response in gestational tissue, leading to preterm labor[12,13]. A recent review and metanalysis[14] showed that women with CIN have a higher baseline risk for preterm birth than women from the general population. Local cervical treatment for CIN 2-3 further enhances the risk that increases also with raising cone depth (and volume). However, a few studies have analyzed how the cervical length varies before and during the subsequent pregnancy after LEEP and whether this correlates to the pregnancy duration at delivery[11]. We, therefore, analyzed the risk for preterm birth in subsequent pregnancies after LEEP for high-grade CIN (CIN 2-3) with respect to the volume of the excision specimen, as well as the cervical length. The aim was to better define the role of the amount of cervical tissue removed on cervical function during the subsequent pregnancy. In addition, we wanted to verify whether other additional risk factors (maternal age, cigarette smoking, ethnicity, vaginal bacteriological infection, cone depths) could affect the risk of premature delivery in women undergoing LEEP.

Patients and Methods

The recruitment was performed from January 1, 2013, to January 31, 2016. The study included all nulliparous women in reproductive age, planned for excisional treatment (LEEP) for CIN 2-3 who wished to have their first pregnancy. All women provided written informed consent for use of personal and clinical data with guarantees of confidentiality. The study was reviewed and approved by the Institutional Review Board (Prot. CE 131/12). Exclusion criteria were: history of previous cervical treatment (one or more previous LEEP/cervical ablation), multiple pregnancies, pregnancies following assisted reproductive technology (ART), patients older than 45 years, any serious illness arises before pregnancy (cardiovascular diseases, diabetes mallitus, infections, hypertension), any pregnancy complications (gestational diabetes, pre-eclampsia, gestational hypertension, diseases of coagulation, placenta previa, placental abruption). Women with no identifiable pregnancy outcome data and women who delivered elsewhere were also excluded. The lengths of the cervix (CL) before treatment (C0) were calculated by two-dimensional transvaginal sonography (2D-TVS). The standardized method of obtaining CL, as described by Berghella et al[15] and Mella et al[16] in the Cervical Length Education and Review (CLEAR) program (Perinatal Quality Foundation, OK, USA), was used. The women were asked to empty her bladder and then the probe was inserted into the anterior fornix of the vagina to obtain a sagittal long-axis view of the cervical canal. Care was taken not to exert pressure on the cervix. The CL was measured from the internal to the external os. At least three measurements were obtained for each dimension and the shortest was used in the analysis. After imaging, the patients underwent excisional treatment by a single experienced colposcopist. Three physicians (one in each study Centre) with expertise in lower genital tract pathology performed the vast majority of LEEPs within the Colposcopy Unit in outpatient setting under local anesthesia. Diathermy loops were chosen according to the area of cervical tissue to remove, and the location of the cervical transformation zone. All excisions were performed under strict colposcopic guidance. When necessary, excision was performed in two steps to ensure that the entire lesion had been removed. Specimens were evaluated by expert pathologists in lower genital tract diseases. The histology reports were retrieved to assess the dimension of the removed tissue. More in detail, the transverse diameter (a), longitudinal diameter (b) and depth (or length) (c) of the cone specimen were recorded. The volume of the surgical specimen was calculated employing the hemi-ellipsoid formula as described by Phadnis et al[17], $1/2 \times 4/3 \times \pi \times a/2 \times b/2 \times c$, postulating that the cone specimen obtained was a hemi-ellipsoid, as the parameters a, b, and c were often unequal. All pregnant women carried out transvaginal sonography (B0 = 6-8 + 6 weeks) to make ultrasound diagnosis of pregnancy, evaluate embryonic cardiac activity, and measure the cervical length. During the first trimester of pregnancy, the due date was calculated. All women had a transvaginal sonography at time B0 (6-8+6 weeks), B1 (16-18 + 6 weeks), B2 (20-22 + 6 weeks), B3 (24-26 + 6 weeks), and B4 (28-32 + 6 weeks). The CL was measured as stated above. All patients with a CL <25 mm before 24 weeks of pregnancy underwent prophylactic cerclage. During the first obstetric examination all women had a vaginal and cervical swab (T0 = 6-8 + 6 weeks). Patients with positive tests performed a specific antibiotic therapy for at least 7

Table I. Sociodemographic and medical characteristics of the 468 pregnant patients previously underwent a single LEEP.

Characteristics	LEEP (n = 468)
Age (years) (mean ± SD)	31.96 ± 5.24
Interval between LEEP and pregnancy (months) (mean ± SD)	12.04 ± 4.67
Gestational age at births (weeks) (mean ± SD)	37.53 ± 2.91
Smoking	63 (13.47%)
Ethnicity Caucasian	415 (88.6%)
African	22 (4.7%)
Asiatic	31 (6.62%)

Data are n (%) unless otherwise specified.

days, and repeated the T1 control swab (10-12 + 6 weeks). Except for T0 and T1 predefined swabs, additional swabs and possible subsequent antibiotic therapies were performed only for symptomatic patients. Those with T0 negative swab underwent, as per guidelines, *Group B Streptococcus* (Group B *Streptococcus* – GBS; *Streptococcus Agalactiae*) research between 35-37 weeks of gestation (T2). On the base of gestational age at delivery, patients have been divided into two groups: term birth (Group 1) and preterm birth (Group 2). Preterm birth was defined as spontaneous labor and delivery between 24 and 37 completed gestational weeks.

Statistical Analysis

A statistical analysis was performed with SPSS version 21.0 (IBM Corp., IBM SPSS Statistics for Windows, Armonk, NY, USA). The Student's *t*-test was used for continuous variables; the χ^2-test was used for categorical variables, as appropriate. Continuous parametric variables were expressed as mean ± standard deviation (SD). Logistic regression was used to evaluate the risk of preterm birth: predictor (or independent) variables were: the patients' age, the volume of the surgical specimen and the cervical length (C0 and B0). A p-value <0.05 was considered statistically significant.

Results

A total of 1435 women who underwent LEEP for CIN 2-3 during the study period were identified. No intraoperative complications, postoperative complications, need for blood transfusions, were recorded. During the study period, 674 of them wanted to get pregnant after LEEP. Of these, 587 (87.09%) got pregnancy. A total of 70 women (11.93%) were lost to follow-up or withdrew from the study and were not reassessed at the first post-treatment visit 6 months later. A total of 49 (9.48%) had spontaneous abortion before 24 weeks and were excluded from the study. The socio-demographic and pertinent medical characteristics of the study population (n=468 women) were reported in Table I. The first vaginal and cervical swab performed during pregnancy (T0) were negative in 81.83% of patients (n=383). Table II shows the infection outcomes of pregnant patients at T0. The most prevalent infections were related to *Candida Albicans, Gardnerella Vaginalis* and GBS. The overall prevalence of preterm birth was globally low (9.4%). A total of 44 cases of preterm delivery were found. Table III shows the comparison of socio-demographic and medical characteristics between woman with term birth (Group 1) and preterm birth (Group 2). More in detail, we found a significantly higher rate of preterm delivery in women with a lower cervical length C0, a higher depth of cone and cone volume, a lower CL from B0 to B4 and with the presence of infections at T0 and T1. Sixteen women with preterm delivery (36.36%) had a caesarean delivery; the rate of caesarean section appeared to be independent from

Table II. Infection outcomes of pregnant patients at T0.

Infection	T0	%
Candida albicans	20	21.51
Gardnerella vaginalis	14	15.05
Mycoplasma	7	7.53
Chlamydia	6	6.45
Candida non albicans	5	5.38
Group B Streptococcus (GBS)	14	15.05
Enterococcus	3	3.23
Gardnerella + Peptococcus	6	6.45
Candida albicans + Penterococcus	3	3.23
Garnderella + Peptococcus + Candida a.	7	7.53
E. Coli	2	2.15
E. Coli + Enterococcus	3	3.23
E. Coli + Candida a.	3	3.23

Table III Comparison of sociodemographic and medical characteristics between woman with term birth (group 1) and preterm birth (group 2).

	Group 1: term birth (n=424)	Group 2: preterm birth (n=44)	*p*-value
Gestational age at delivery (week)	38.01 ± 1	31.95 ± 2.7	< 0.0001
Age (years)	31.75 ± 5.22	33.93 ± 5.31	0.01
Interval between LEEP and pregnancy (months)	12.04 ± 4.64	12.0 ± 5.0	0.96
Smoking	60 (14.15%)	3 (6.61)	0.17
Caucasian	376 (88.67%)	39 (88.63%)	0.18
Asiatic	30 (7.07%)	1 (0.22%)	
African	18 (4.24%)	4 (9.09%)	
Cervical length before treatment (C0) (cm)	4.30 ± 0.14	4.23 ± (0.17)	0.01
Cone volume (cm3)	1.02 ± 0.52	1.31 ± 0.61	0.001
Depth of cone (cm)	1.21 ± 0.13	1.27 ± (0.17)	0.048
Cervical lenght (cm)			
B0	3.08 ± 0.19	2.96 ± 0.29	0.01
B1	2.96 ± 0.21	2.74 ± 0.30	< 0.0001
B2	2.83 ± 0.22	2.56 ± 0.29	< 0.0001
B3	2.77 ± 0.23	2.29 ± 0.52	< 0.0001
B4	1.63 ± 0.29	1.69 ± 0.45	< 0.0001
Infection outcomes			
Infections at T0	49 (11.56%)	36 (81.8 %)	< 0.0001
Infections at T1	6 (1.42%)	14 (31.82 %)	< 0.0001

Data are expressed in mean ± SD unless otherwise specified.

the gestational age at birth. In particular, women at term birth and at preterm delivery had similar rate of caesarean section (25.71% *vs.* 36.36%, $p = 0.12$). However, data on caesarean indication are not available. Group 2 shows a high prevalence of *C. Albicans* infections (alone or in association with other microorganisms) (29.55%), a high prevalence of *G. Vaginalis* infections (alone or in association with other microorganisms) (29.55%) and a high prevalence of *Mycoplasma* and *GBS* infections (both 9.09%). After specific antibiotic therapy (T1), in group 2 persistent infection of *G. Vaginalis* (alone or in association with other microorganisms) (20.46%), *Mycoplasma* (9.09%), *Chlamydia* (2.27%) emerged. Table IV shows the multivariable logistic regression of risk factors for preterm birth confirming the previous significant associations between volume of the specimen and infection outcomes at T1 and risk for preterm birth.

Table IV. Multivariable logistic regression of risk factors of preterm birth.

	95% C.I. for EXP (B)	*p*-value
Age	1.066 (0.996-1.140)	0.064
Cone volume	2.080 (1.039-4.166)	0.039
C0	0.071 (0.001-3.621)	0.187
B0	0.142 (0.010-2.036)	0.151
T1	76.963 (21.517-275.285)	0.0002

Data are expressed in mean ± SD unless otherwise specified.

Discussion

The exact etiology of the pregnancy-related morbidity after cervical excisional treatment for CIN is not completely understood. Nevertheless, the depth (or length) and the volume of cervical tissue excised have been shown to be directly related to the risk for preterm birth. Our findings showed a significantly higher rate of preterm delivery in women with a lower cervical length before the treatment and in the subsequent pregnancy, and in women with a higher depth and volume of cone or in the presence of infections in the first trimester of gestation. After excluding for confounding factors (Table IV), women with preterm birth showed a higher cone volume excised. This agrees with a large recent metanalysis[18] that clearly shown that the risk for preterm birth is directly related to the cone dimensions (depth/volume), and progressively increase with increasing cone depth ("dose effect"). The volume of the cone is therefore essential. Our results reaffirm the concept of modulation of conization, which must always be carried out under strict colposcopic guidance. A recent meta-analysis suggested that LEEP is associated with a higher risk for preterm birth, although this risk was attenuated if compared to women with cervical dysplasia but no LEEP[18]. This suggests that the LEEP itself may not be the causative agent, but instead the combination of LEEP and other confounders together may augment the risk for preterm birth[19]. Among these factors, vaginal infections during pregnancy in women with a history of LEEP may be associated with an increased risk for preterm birth, compared with women with no history of LEEP[19]. Another aspect to be evaluated is presence of vaginal infections at vaginal and cervical swabs performed during pregnancy following LEEP. There is a well-established association between infection and risk of abortion or preterm birth. Healthy vaginal microbial communities are dominated with low bacterial diversity, while high diversity bacterial populations often characterize vaginal dysbiosis. The increase of this biodiversity increases the risk of infections, preterm deliver, persistence and development of cervical cancer[20]. Romero et al[21] investigated whether the vaginal microbiota composition in pregnant women who had a spontaneous preterm delivery is different from that of women who deliver at term: they concluded that there is no difference between the two groups. Our results show that the presence and the persistence of vaginal infections after specific antibiotic therapy determine an increased risk for preterm birth in patients who underwent LEEP. One potential mechanism for this association could be the loss of antimicrobial action of the endocervical mucus normally present at the transition zone. The cervical mucus is thought to provide both a mechanical and chemical antimicrobial barrier[19,22-24]. Our data show that what is most important is the persistence of infection (even after targeted antibiotic therapy), probably linked to an alteration of the bacterial flora and a higher susceptibility to re-infection in women undergoing LEEP[25]. The most common infections found were *C. Albicans*, *G. Vaginalis*, *GBS* and *Mycoplasma*.

Conclusions

The role of bacterial infections in determining a preterm birth is already known in the literature, while the importance of treating *Candida* infections has recently been reaffirmed in a large meta-analysis[26]. The findings of our analysis were not unexpected at all, but two of the strengths of this study are the large number of pregnancies after LEEP considered and the prospective nature of the study. Furthermore, all the excisional procedures were performed at our Colposcopy Units using a standardized technique. In addition, the meticulous gathering of data through medical chart review enabled the collection of data involving multiple confounding factors and decreased recall bias. The study suggests that in women undergone LEEP it may be essential to know the volume of excised cone for better informing the patients about their risk for preterm birth. Moreover, in this group of patients the use of vaginal swabs and of CL measurement can be useful for identifying women at risk for premature birth and for implementing any therapeutic strategies.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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